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Insect diversity

A Burrowing Mayfly, *Anagenesia minor* (Eaton) in the Mekong River

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Abstract

An annual massive swarm of burrowing mayfly, *Anagenesia minor* (Eaton) occurred in hot season along the bank of the Mekong River. This occurrence was well known by Thai local people. Objective of this study was to investigate some biological aspects of *A. minor*. Nymphs were sampled by a D-frame dip net (450 μm mesh) from mud, sand, and clay along the shoreline of the Mekong river reaches between Nakhon Phanom and Mukdahan provinces, northeast of Thailand during April 13～14, 2007. Adults were collected by hand picking from the massive swarm on April 16, 2007. The result showed that it was successful to associate nymphs with adults by male genitalia. The highest density of nymphs sampled were in site I which is clean site and lowest density sampled was in site III which is the polluted site. Body sizes of female nymphs and adults were larger than those males. Abdomens of gravid female nymphs were full of numerous eggs. Mean of fecundity ± SD was 3,824.63 ± 1,836.00 (n=8). Sex ratios between male: female nymphs and adults were 1.32 : 1 and 1.85 : 1, respectively. Gut content analysis of nymphs (n=5) resulted mainly of minerals (90%), detritus (9.99%) and diatoms (0.01%).

Key words: *Anagenesia minor*, burrowing mayfly, Mekong River

Introduction

Mayfly belonging to the genus *Anagenesia* was established by Eaton (1883). So far, there were 15 species described under this genus (Kluge, 2004; Barbel-James *et al.*, 2008). Fourteen species, *Anagenesia albescens* (Demoulin), *A. ampla* (Eaton), *A. birmanica* (Navas), *A. javanica* (Eaton), *A. lata* (Walker), *A. leucoptera* (Navas), *A. lontona* (Hafiz), *A. minor* (Eaton), *A. nana* (Demoulin), *A. nanoides* (Demoulin), *A. picta* (Gravely), *A. robusta* (Eaton), *A. spodiocephala* (Demoulin), and *A. tenera* (Eaton), were distributed in the Oriental region whereas only one species, *A. paradoxa* (Buldovsky), was found from the Palaearctic region (Kluge, 2004). The burrowing mayfly *A. minor* was widely distributed over the Indian Empire, Burma, and spread over to Borneo and Sarawak of Indonesia (Chopra, 1927). This species was also distributed along the Mekong river between Thailand and Lao People's Democratic Republic. Massive swarm emergence of of *A. minor* is reported annually in hot season especially April at Mukdahan province, northeast of Thailand. This phenomenon caused nuisance to the local residents living along the river and interference with road traffic (Komchadluek News, 2007; Mukdahan Health Care, 2007). The distribution and morphology of adult of *A. minor* have been reported (Chopra, 1927), however some biological aspects of the nymphs are not well known. The objectives of this study were to determine density, body size, sex ratio, fecunfity and gut content of mature nymphs and also density and body size of adults.

Methods

Study Area

The study area consisted of five sampling sites situated along the Mekong River bank 127 km between Nakhon Phanom and Mukdahan Provinces, northeast of Thailand. The locations of sampling sites are shown in Fig. 1. Site I is adjacent to Tha U Then water supply station, Tha U Then District, Nakhon Phanom province. Site II is Art Samart village, upstream Muang District, Nakhon Phanom province. Site III is near Thad Phanom District with intensive activities such as pier, fish culture, restaurants and recreation. Site IV is near Ban Nam Kam, Nakhon Phanom province. Lastly, site V is upstream the Thai-Laos Friendship Bridge, Mukdahan province. Substrata of all sampling sites were mud, sand, and clay.

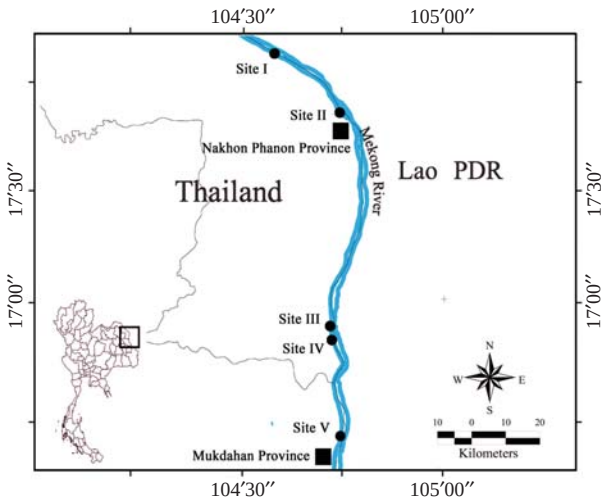

Fig. 1. Location of sampling sites.

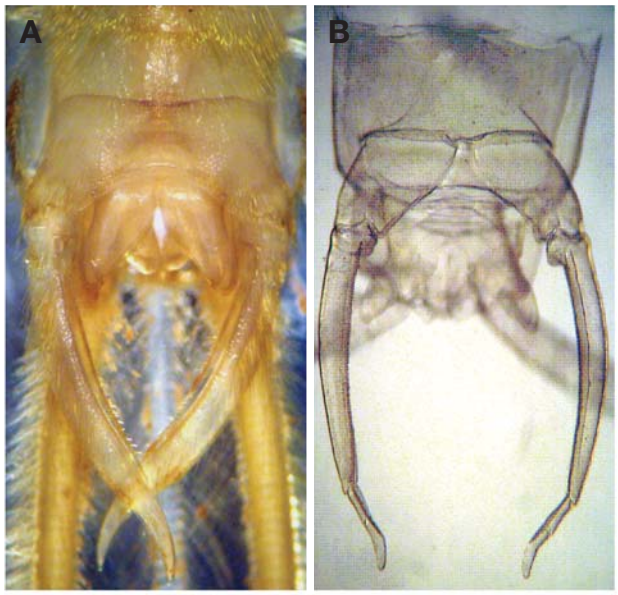

Fig. 2. Genitalia of (A) *A. minor* nymph, (B) adult.

Sampling and Laboratory Procedures

Benthic samples were collected on April 13～14, 2007 using a D-frame dip net (450 µm mesh, 20 × 20 × 30 cm^3). Five samples were collected per site. Invertebrates were stored into labeled vials containing 70% ethanol. Individuals were later sorted, identified and counted for density in the laboratory. In addition, mayfly adults were collected on April 16, 2007 (19:30～20:00) from a massive swarm falling down on the road by hand picking at Ban Bang Sai Noi, 300 m downstream site V. Specimens were preserved in 70% ethanol and transported to the laboratory. Nymphs and adults were associated using male genitalia. The identification keys to species were according to Gravely (1920); Lestage (1923); Chopra (1927); Hafiz (1937); Kimmins (1960); Demolin (1965). Numbers and sexes of nymphs and adults were counted under stereo microscope to calculate density and sex ratios.

Body Sizes

Width of head capsule, body length from the frontal process of head to the end of 10th abdominal segments, widths of the pronotum and mesonotum of nymphs were measured (n=12). The width of head, the lengths of body, fore wing, and hind wing of adults (n=10) were also measured using an ocular micrometer under the stereo microscope (10～70 ×).

Fecundity

Fecundity estimates were based on gravid females nymphs (n=8) from preserved samples. Ovaries of some gravid specimens were stained with methylene blue to facilitate egg counting.

Gut Analysis

Nymphs with dark wing pads were selected as the mature nymphs as described by Edmunds and McCafferty (1988). Guts of the mature nymphs (n=5) were isolated. The gut contents were diluted in glycerin and then placed on microscopic slides (one nymph/slide) according to the methods of Landolt *et al.* (1995). The diet contents were determined to the lowest possible taxa level and quantified as a percentage of the total stomach content per sample. The percentage of diet composition has been obtained by pooling the data of individual nymph.

Data Analysis

T-test was used to determine the significant differences of body sizes of females and males. Pearson correlation was used to analyze egg numbers and body sizes.

Results and Discussion

From the present study, it was successful to associate nymphs and adults by male genitalia (Fig. 2) of *A. minor* (Eaton) (Figs. 3, 4).

Density

As shown in Table 1, the highest density of nymphs occurred at site I (34.67%), followed by site II (33.50%), site IV (18.75%), and then site V (11.51%). The lowest density was found at Site III (1.55%), which may be due to intensive activities such as sand dredging services, pier, fish cultures, restaurants and recreation which caused water pollution and disturbed microhabitats of benthos (Edsall *et al.*, 2005).

Fig. 3. *A. minor* nymphs (scale bar=5 mm).

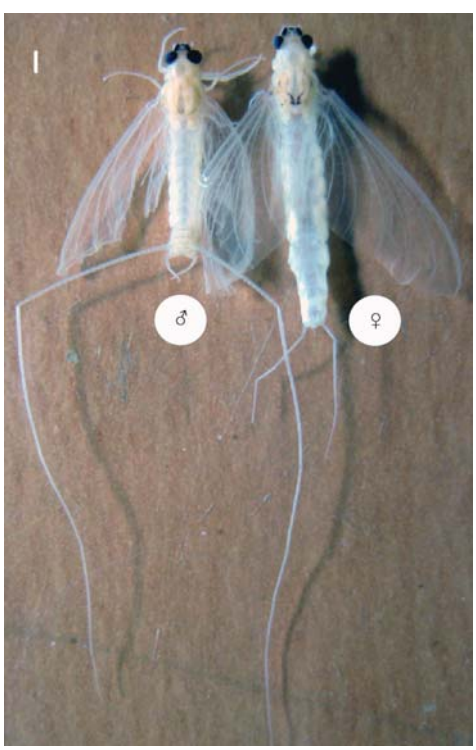

Fig. 4. *A. minor* adults (scale bar=2 mm).

Table 1. Density of *A. minor* nymphs along the Mekong River

Sites	No. of nymphs	Density (Ind. m^{-3})	No. of ♂ : ♀	Sex ratio ♂ : ♀
I	268	2,138	26 : 22	1.18 : 1
II	259	2,066	79 : 51	1.54 : 1
III	12	96	4 : 2	2.00 : 1
IV	145	1,157	30 : 28	1.07 : 1
V	89	710	13 : 13	1.00 : 1
Total	773	1,234	152 : 115	1.32 : 1

Table 2. Range (Mean ± SD) of body sizes of *A. minor* nymphs from the Mekong River

Body sizes (mm)	♂ (n=10)	♀ (n=12)
Head width	3.0-3.2 (3.09 ± 0.7)**	3.2-3.4 (3.28 ± 0.9)**
Body length	21.1-29.0 (27.06 ± 28.1)	28.8-32.6 (30.75 ± 13.7)
Pronotum width	3.6-4.0 (3.8 ± 1.2)*	3.9-4.3 (4.14 ± 1.3)*
Mesonotum width	3.8-4.1 (3.99 ± 0.8)**	4.2-4.7 (4.35 ± 1.6)**

**$p < 0.01$, *$p < 0.05$

Table 3. Range (Mean ± SD) of body sizes of *A. minor* adults from the Mekong River

Body sizes (mm)	♂ (n=10)	♀ (n=10)
Head width	22.0-26.0 (24.05 ± 1.42)	21.0-29.0 23.9 ± 2.23
Body length	131.0-185.0 (161.6 ± 17.66)	147.0-210.0 (170.3 ± 21.40)
Fore wing length	131.0-140.0 (125.40 ± 7.90)**	140.0-175.0 (154.10 ± 12.04)**
Hind wing length	49.0-68.0 (57.30 ± 5.20)*	57.0-75.0 (64.30 ± 6.29)*

**$p < 0.01$, *$p < 0.05$

Body Sizes

Body sizes of the nymphs are presented in Table 2. It showed that the head and mesonotum widths as well as the pronotum widths of female nymphs were significantly larger in females than in males ($p < 0.01$ and $p < 0.05$, respectively). However, body lengths of the female nymphs were not significantly different from those of male nymphs ($p > 0.05$).

As presented in Table 3, the head widths and the body lengths of female adults were not significantly different from those of male adults ($p > 0.05$). Whereas the fore wing lengths of the female adults were highly significant longer ($p < 0.01$), and the hind wing length of the female adults were significantly longer ($p < 0.05$) than those of male adults. However,

Table 4. Pearson correlation and p value of egg numbers and body sizes of nymphs

Body sizes (mm)	r	p
Head width	0.81	0.013
Body length	0.56	0.149
Pronotum width	0.65	0.079
Mesonotum width	0.85	0.007

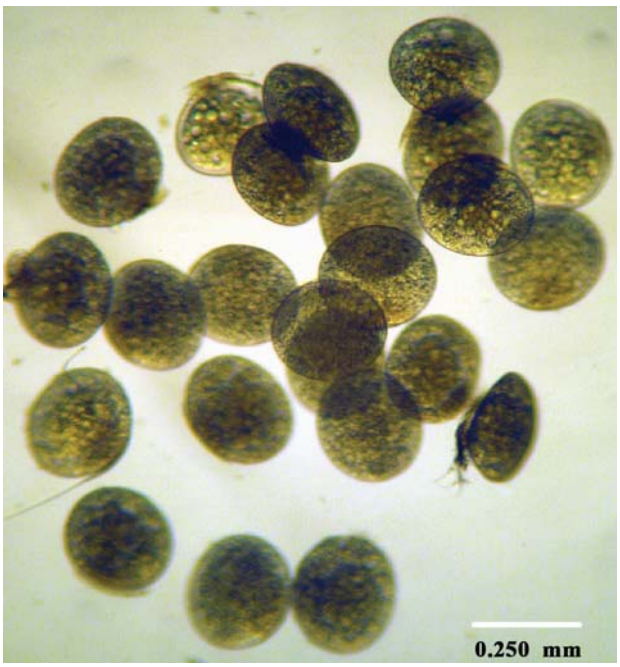

Fig. 5. Eggs of *A. minor* nymph.

the body measurements of both female nymphs and adults were larger than the male nymphs and adults. This result is similar to the study done by Chopra (1927) showing that *A. minor* adult females are larger than the adult males.

Sex Ratio

Sex ratios between male: female nymphs were 1.32 : 1 (152/115), whereas the sex ratios of male:female adults were 1.85 : 1 (280/151) (Table 1). It showed that ratios of adult males were higher than females, which may be due to the collecting time, 19:30∼20:00 wherein more male adults usually emerged. This result is similar to the study done by Watanable *et al.* (1989) reporting that more male subimagoes will emerge 1-2 hours earlier than the female subimagoes.

Fecundity

Ranges and mean ± SD of eggs numbers counted from gravid female nymphs were 1,691∼7,197 and 3,824 ± 1,836, respectively. It was found that body sizes of female nymphs were strongly correlated with number of eggs (Table 4). Eggs were oval-shaped with numerous globular yolk granules scattered (Fig. 5) which was similar to the report of Chopra (1927).

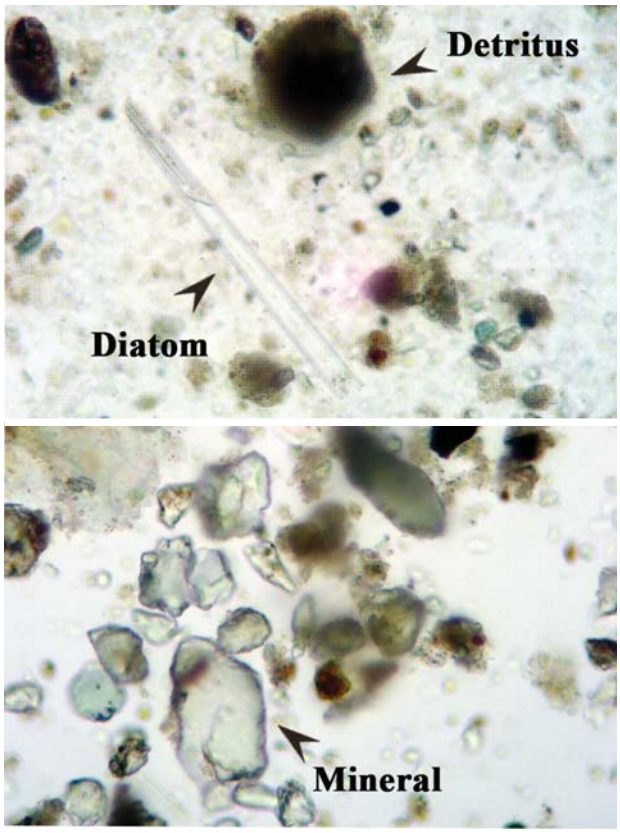

Fig. 6. Food contents of *A. minor* nymph.

Gut Analysis

As shown in Fig. 6, the main food items of the mature nymphs (n=5) were minerals (90%), followed by detritus (9.99%) and then diatoms (0.01%), which was corresponded to the substrate. Gut analysis showed that *A. minor* nymphs are collector-gatherers which was similar to the study of Edmunds and Waltz (1996) showing that burrowing mayflies (superfamily Ephemeroidea included *A. minor*) are collectors-gatherers.

This is the first report of some biological aspects of *A. minor* nymphs from Thailand.

Acknowledgements

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